

The goal of this project is to assess the compensatory role of MNA/COX-2/PGI2 pathway in the development of endothelial dysfunction associated with NO deficiency as well as the evaluation of anti-platelet effects of pharmacological stimulation of this pathway in various degree of NO-deficiency in mice.

1-methylnicotinamide (MNA) is a metabolite of nicotinamide (NA) synthesised mainly by nicotinamide-N-methyltransferase (NNMT) present in the liver. Exogenous MNA (1-methylnicotinamide) exerts anti-thrombotic and anti-inflammatory effect through activation of COX-2/PGI2 pathway. The concentration of MNA in inflammatory states such as atherosclerosis or hepatitis is elevated what indicates that this molecule can counteract the development of endothelial dysfunction associated with those pathologies. Prostacyclin (PGI2) is a lipid mediator produced from arachidonic acid (AA) via cyclooxygenase-2 (COX-2) and possesses antithrombotic, antiproliferative and anti-inflammatory properties.

We hypothesize, that endogenous MNA is the regulator of COX-2/PGI2 pathway activity, thus the activation of the compensatory in various degree of NO-deficiency in mice. NO is mainly produced by the vascular endothelial cells and exerts vasodilation of vascular smooth muscle cells, protects the endothelium from platelets adhesion and aggregation and prevents to atherosclerosis development.

This project aims to verify this hypothesis using NO-deficient mice with hypertension induced by L-NAME which is an inhibitor of all isoforms of NO synthases. To confirm our thesis, we will evaluate the influence of impaired NO bioactivity on the action of MNA/COX-2/PGI2 system by the quantification of MNA and its metabolites (2-Met-PY, 4-Met-PY), evaluation of eicosanoids profile in urine and plasma specimens and also by platelets activity analysis assessed in whole blood by ex vivo dynamic TXB2 generation assay. The changes in platelets activity analysed by ex vivo dynamic TXB2 generation assay in whole blood combined with alternation in MNA and PGI2 synthesis in NO-deficient mice during progression of hypertension and after pharmacotherapy using MNA or MNA and NO donors will allow to assess the importance of NO and COX-2/PGI2 pathways in the regulation of platelets activity in different stages of endothelium-derived NO bioavailability impairment.

The project is also concentrated on the development of bioanalytical method for quantification of selected eicosanoids in mice urine and plasma applying LC/MS/MS technique as well as method validation including following parameters: linearity range, precision, accuracy, matrix effect, analytes stability. Additionally, the optimization of plasma samples clean-up procedure will be performed.

Moreover, based on the results we will attempt to evaluate the PK/PD model encompassing the integration of pharmacokinetics, biochemical and pharmacodynamics parameters which describe the biological effect of studied substances and antiplatelet activity of two considered pathways - NO and COX-2/PGI2.

We believe that the realization of this project allows us to provide more details about compensatory mechanism of MNA/COX-2/PGI2 activated in various degree of NO-deficiency in mice. Thanks to the new knowledge about changes in platelets activity as well as MNA, eicosanoids and NO profile in different stages of hypertension and NO-deficiency we will be able to support our thesis that MNA elicits the compensatory effect mediated by COX-2/PGI2 during the development of endothelial dysfunction. We want to show, that MNA change the balance between NO and COX-2/PGI2 pathways via PGI2 production in L-NAME-induced hypertension. The clarification of interactions between NO and COX-2/PGI2 could have a great significance in understanding of MNA/NO/PGI2 interactions in hypertensive NO-deficient mice and may contribute to the counteraction of endothelial dysfunction associated with hypertension resulting in reduction of cardiovascular episodes and mortality

This interdisciplinary project provides an opportunity not only to develop a new bioanalytical method (eicosanoids profiling using LC/MS/MS technique) but also to discover new research tools (PK/PD modeling). However, the most important advantage of this project is the possibility of confirmation of anti-thrombotic activity of MNA via compensatory role of MNA/COX-2/PGI2 mechanism in endothelial dysfunction and various degree of NO-deficiency in mice.